



GEIS Respiratory Disease Surveillance Newsletter

DoD Center for Deployment Health Research
Naval Health Research Center, San Diego



Issue 6

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The DoD Center for Deployment Health Research at the Naval Health Research Center (NHRC) serves as the Navy hub for the DoD Global Emerging Infections System (GEIS). Through this system, NHRC collaborates with numerous institutions to conduct surveillance for respiratory pathogens, including adenovirus, influenza, respiratory syncytial virus, parainfluenza, *Bordetella pertussis*, *Streptococcus pyogenes*, and invasive *Streptococcus pneumoniae*. For more detailed information about NHRC DoD-GEIS projects, please visit our website at <http://www.nhrc.navy.mil/geis>.

SARS Testing at NHRC

In June 2003, NHRC was designated as a Laboratory Response Network (LRN) site for Severe Acute Respiratory Syndrome (SARS). Facilities with suspected cases of SARS can send specimens to NHRC for screening, where they will be tested using reagents and protocols provided by CDC. SARS-positive cases will be reported through the appropriate channels and specimens forwarded to CDC for confirmation.

Current Study Updates

Pneumococcal Vaccine Trial

In collaboration with Centers for Disease Control and Prevention, the Mayo Clinic and Foundation, and Wyeth Pharmaceuticals, NHRC and 4 recruit training sites are conducting a double blind, placebo-controlled trial of a 23-valent pneumococcal vaccine to assess the vaccine's clinical effectiveness among the military trainee population. The study is currently underway at Fort Jackson, Fort Leonard Wood, NRTC Great Lakes, and MCRD Parris Island. Enrollment was completed at all sites and active surveillance for pneumonia while participants are still at basic training is in its final stages. The participants are later passively followed (by tracking inpatient and outpatient military medical databases) for pneumonia and acute respiratory disease after recruit training until October 2003. To date, study site personnel have enrolled more than 157,000 recruits with an enrollment rate of approximately 75 percent.

Evaluation of PCR Testing Using Room Temperature Specimens

One of the biggest challenges the Febrile Respiratory Illness (FRI) sites face is keeping viral specimens frozen at ultra-low temperatures for long periods before and

during shipping. A sub-study at Fort Jackson was conducted to evaluate the performance of room temperature specimens in comparison with frozen viral culture. The study, in collaboration with researchers at the Armed Forces Institute of Pathology, uses PCR techniques to test for adenovirus and influenza among specimens that have been stored at room temperature. If these tests prove successful, FRI surveillance could be simplified and expanded to regions where freezing specimens is not feasible. The first specimens collected during the 2001-2002 flu season yielded promising results, and specimens collected during the 2002-2003 season were tested with newer methods. Preliminary analyses show promising results.

Febrile Respiratory Illness (FRI) in a Mexican population

In collaboration with the Mexican Institute of Public Health Services, FRI surveillance was conducted at a community clinic in Ensenada, Mexico from December 2001 through March 2003.

Among the 108 specimens collected, 100 have laboratory results. Of these, 15 were positive for influenza (9 type A and 6 type B); 7 were positive for adenovirus; 5 were positive for parainfluenza; 3 were positive for respiratory syncytial virus; 3 were positive for herpes simplex virus-type 1; 1 was positive for enterovirus; and 69 were negative for viral pathogens. One of the first North American influenza cases of the 2002-2003 influenza season was identified in this population; the influenza B isolate recovered by NHRC was a vaccine-preventable strain.

This work demonstrates the ability of the US Navy Respiratory Disease Laboratory at NHRC to collaborate with our Mexican neighbors on a limited respiratory disease surveillance project.

Association Between Adenovirus Infections Among Military Personnel and the Development of Obesity

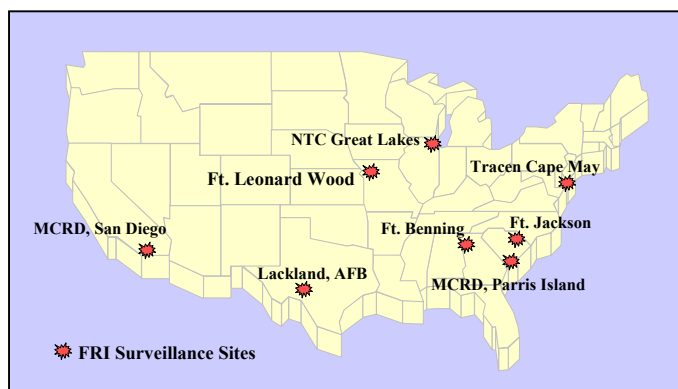
This case control study, which began in October 2001, investigates if an association exists between weight control problems (obesity) and adenovirus-36 exposure in a population of active-duty Navy personnel. The study, which is being conducted at the Clinical Trials Center and at other military commands in San Diego, is currently enrolling cases (individuals with a Body Mass Index (BMI) of >30) and controls (BMI <25).

Laboratory Based Surveillance for Febrile Respiratory Illness Aboard Floating Platforms

Until now, laboratory supported surveillance for respiratory pathogens aboard U.S. military ships in the Pacific Rim has been sparse, despite viral illness outbreaks repeatedly occurring in these settings. This study, currently underway, examines respiratory illness aboard floating platforms home based in the San Diego area. Viral throat culture specimens are obtained from shipboard personnel presenting with FRI symptoms, and tested here at NHRC to determine FRI etiology aboard ships, both at home and while deployed.

Real-time Diagnostic Capabilities on Aircraft Carriers – Real-time PCR techniques for influenza and adenovirus detection have been deployed for use on an aircraft carrier. This capability will utilize existing LightCycler® equipment aboard aircraft carriers. Routine use of this equipment, while testing for respiratory pathogens, will help to maintain technical proficiency.

Febrile Respiratory Illness Surveillance



Current Progress – Febrile Respiratory Illness surveillance continues at eight military training sites. From April 2002 to February 2003, 2,159 specimens were collected from trainees presenting with symptoms matching the case definition of FRI (oral temperature $\geq 100.5^{\circ}\text{C}$ and cough or sore throat, or any case of radiographically confirmed pneumonia). Specimens are tested for adenovirus, influenza A and B, respiratory syncytial virus (RSV), and parainfluenza 1, 2 and 3. The specific trends observed for each of these viruses are described in the following sections.

FRI Specimens Tested per Site June 1998 – February 2003

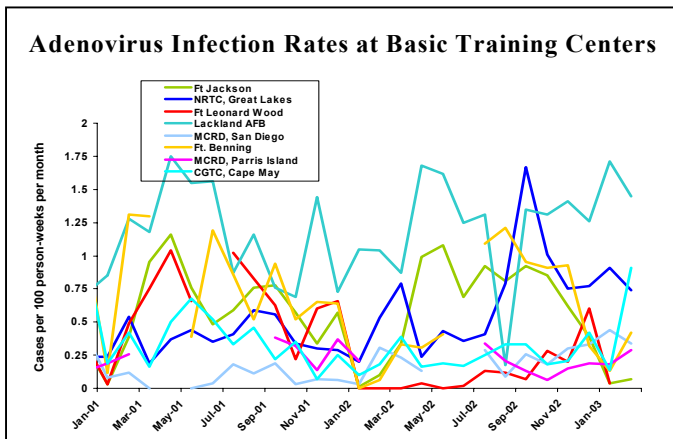
<u>SITE</u>	<u>SPECIMENS TESTED</u>
Fort Benning	1798
Fort Jackson	3417
Fort Leonard Wood	1571
NRTC, Great Lakes	1541
MCRD, San Diego	823
MCRD, Parris Island	465
Cape May	618
Lackland AFB	1309
Total	11,542

Geographic Trends – Since January 1, 2002, five of the eight surveillance sites have reported FRI rates above the epidemic threshold, which is defined as 1.5 cases per 100 trainees per week. The distribution of viral test results by site demonstrates that adenovirus continues to be responsible for the majority of FRI at all surveillance sites.

Temporal Trends – During the period of time between March 2002 and February 2003, FRI rates showed no discernable seasonal trends, as epidemics occurred at various times throughout this period.

Adenovirus

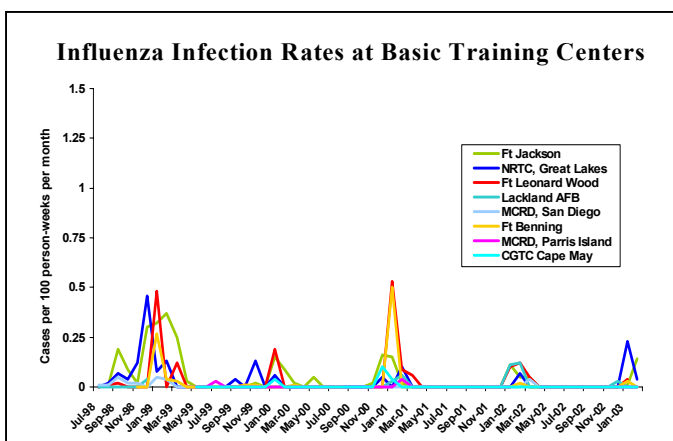
Current Progress – In the absence of vaccine, adenovirus remains the leading cause of FRI among trainees. Sixty-three percent of the 11,542 throat cultures collected for the FRI study between June 1998 and February 2003, and 1,571 (72.8%) of the 2159 collected between April 2002 and February 2003, tested positive for adenovirus. Approximately 98% of all adenovirus isolates collected to date have been type 4.



Geographic Trends – From April 1, 2002 to February 28, 2003, the amount of FRI morbidity caused by adenovirus remained high at all sites, ranging from 32.4% at Fort Leonard Wood, to 85.8% at Lackland AFB. Please see previous chart for adenovirus infection rates by site.

Influenza

Current Progress – Of the 2,159 FRI specimens collected between April 2002 and February 2003 from a highly vaccinated recruit population, 24 (1.1%) have tested positive for influenza. Of all the specimens collected from the start of this study, 451 out of 11,542 (3.9%) have been positive for influenza, with 2.9% identified as type A and 1% as type B. Ill trainees who were not vaccinated against influenza were more than 5 times more likely to be influenza-positive (OR= 5.3, 95% CI 4.2-6.7) than those who did receive the vaccine.



Geographic Trends – Our surveillance data demonstrate that morbidity among trainees caused by

infection with influenza A or B varies by training site location. Please see accompanying chart for influenza infection rates by basic training site location.

Temporal Trends – Influenza activity occurred only during the winter months in 2002-2003, during the typical “flu-season”. These recent influenza rates among military recruits were somewhat low in comparison previous flu seasons.

Other FRI Study Pathogens

Of the 11,542 throat cultures tested thus far under the FRI study, 21 (0.2%) have been positive for RSV and 92 (0.9%) have been positive for parainfluenza 1, 2, or 3. Of the 2,159 specimens collected from April 2002 to February 2003, 18 (0.7%) have been positive for parainfluenza 1, 2, or 3; none of these samples were positive for RSV.

Streptococcus pyogenes Surveillance

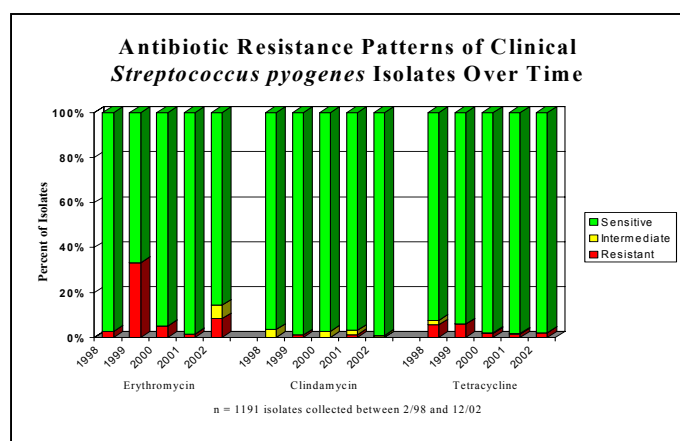


Current Progress – As demonstrated by a GAS pneumonia outbreak at MCRD San Diego in December 2002, *Streptococcus pyogenes* (Group A streptococcus [GAS]) continues to be a threat to the health of military trainees. From the start of this surveillance in February 1998 through February 2003, 1,297 (416 in the last year) GAS-positive clinical isolates were collected from laboratories at 8 military training sites. Antibiotic resistance testing was performed on all isolates, and emm-gene sequencing was performed on a set of randomly selected samples.

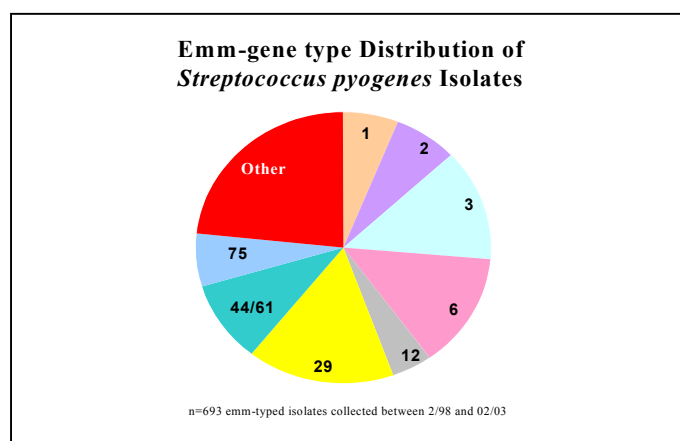
Antibiotic Resistance – Among the specimens tested, *S. pyogenes* maintains 100% susceptibility to the antibiotics penicillin and vancomycin. One hundred and forty-one (10.9%) of the 1297 isolates collected demonstrated full or partial resistance to erythromycin,

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60 (4.6%) to tetracycline, 27 (2.1%) to clindamycin, and 6 (0.5%) to levofloxacin. Sixteen (1.2%) of the isolates were resistant to both erythromycin and tetracycline. Isolates from female trainees showed a similar proportion of erythromycin resistance as compared to male trainees (11.1% and 9.6%, respectively). Temporal trends in antibiotic resistance among *S. pyogenes* isolates collected to date demonstrate no discernible pattern, as shown in the following chart.



Emm-gene Types – As of May 2003, emm-gene sequencing had been performed on 693 randomly selected samples. The most common emm types were 29 (16.2%), 6 (13.7%), 3 (13.1%), 44/61 (9.7%), 2 (6.9%), 75 (6.3%), 1 (6.2%), and 12 (4.5%). These eight emm-gene types made up more than 76% of all the typed isolates.



Resistance by Emm-gene type – Erythromycin resistance varied by emm-gene type, with Type 75 demonstrating the most resistance of all emm-gene typed isolates (64% were fully resistant). Most (75%) of these Type 75 isolates came from trainees at Lackland AFB,

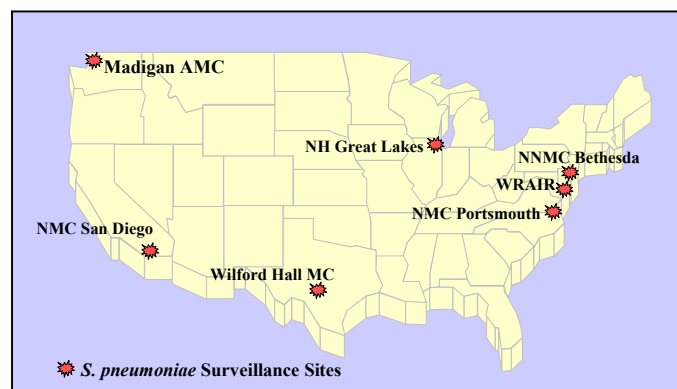
TX. A manuscript of this work is currently under review at the *Journal of Clinical Microbiology*.

Geographic Trends – *S. pyogenes* isolates from military trainees continue to maintain high susceptibility to many commonly prescribed antibiotics including tetracycline, erythromycin and clindamycin, and 100% susceptibility to penicillin, and vancomycin. However, we continue to observe an unequal geographic distribution of erythromycin resistance at the sites. Resistance to erythromycin differed between sites, with Lackland AFB having the highest resistance rate (29.1%). All of the erythromycin resistant isolates from Lackland AFB were fully resistant.

***S. pyogenes* Isolates Received per Site
February 1998 to February 2003**

<u>SITE</u>	<u>ISOLATES RECEIVED</u>
NRTC, Great Lakes	277
MCRD, Parris Island	485
MCRD, San Diego	19
Fort Jackson	4
Fort Knox	23
Fort Leonard Wood	162
Fort Sill	196
Lackland AFB	110
Total	1,297

***Streptococcus pneumoniae* Surveillance**



Current Progress – Invasive clinical isolates continue to be collected from 7 military medical centers across the nation. Three hundred and sixty-eight isolates have been collected thus far, 21 within the last year.

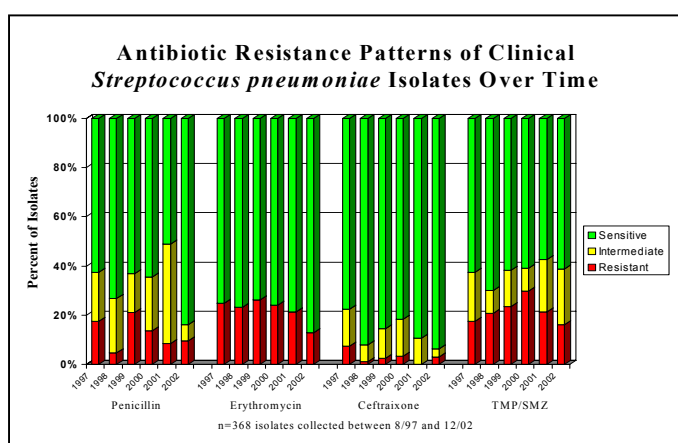
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Geographic Trends – Among sites with an adequate number of specimens, penicillin resistance was relatively consistent, ranging from 30.6% at Madigan AMC to 48.6% at Walter Reed AIR.

Antibiotic Resistance – One hundred and twenty-five (34%) of the 368 isolates collected demonstrated full or partial resistance to penicillin, and 80 isolates (21.7%) exhibited multiple resistance to three or more antibiotics. Penicillin resistance levels among males (35.5% resistant) and females (31.8% resistant) were similar. As

Continued from page 4

shown in the graph below, temporal trends in antibiotic resistance among *S. pneumoniae* isolates collected to date does not demonstrate a discernible pattern.



Resistance by Serotype – Penicillin resistance differed by serotype, with types 19 (54%), 9 (50%), and 6 (48%) demonstrating the most resistance. These three serotypes accounted for more than 62% of all penicillin resistance among the serotyped isolates, though they only comprised 43% of the total number of serotyped isolates.

***S. pneumoniae* Isolates Received per Site August 1997 to February 2003**

<u>SITE</u>	<u>ISOLATES RECEIVED</u>
NRTC Great Lakes	4
NMC, San Diego	99
NMC, Portsmouth	2
NNMC, Bethesda	17
Walter Reed AIR	37
Wilford Hall MC	85
Madigan AMC	124
<u>Total</u>	<u>368</u>

***Bordetella pertussis* Surveillance**

Current Progress – Surveillance of *Bordetella pertussis* is currently underway at four military recruit training sites: MCRD - San Diego, Ft. Benning, Ft. Leonard Wood, and NRTC Great Lakes. Site personnel have enrolled 292 subjects to date. Of these, 147 specimen sets have been tested using 3 different methods. These specimens were collected from recruits meeting the case definition for pertussis, which is more than seven days of coughing with symptoms consistent with a respiratory infection. Preliminary results show that at least one (0.6%) of the specimens tested positive by culture, 9 (6.1%) were positive by PCR, and 3 (2%) tested positive by serology (seroconversion).

***B. pertussis* Specimens Received per Site June 2000 to February 2002**

<u>SITE</u>	<u>SPECIMENS RECEIVED</u>
MCRD San Diego	143
Fort Leonard Wood	80
Fort Benning	40
NRTC Great Lakes	29
<u>Total</u>	<u>292</u>

Expanding Projects in Molecular Epidemiology

Sequencing of Adenovirus Vaccine- Breakthrough Samples

Through respiratory surveillance, NHRC has discovered that from 1996 to 1999 several individuals who received the adenovirus vaccine subsequently became ill with adenovirus 4 or 7 infection. It is important to understand why these vaccinated individuals became ill. Was it simply a poor immune response, or was there something different about the virus that allowed it to “break through” the vaccine? Research by Drs. Dean Erdmann, Lita Crawford-Miksza, and David Schnurr has shown significant variation in the epitope-coding portions of the hexon gene. NHRC, in collaboration with Luke Daum at Brooks Air Force Base and Dr. David Schnurr at the California Department of Health Services, has sequenced and phylogenetically analyzed the hexon gene of each breakthrough strain to determine if genetic drift contributed to these break-through infections. A manuscript on this work is currently under review.

Multiplex-PCR Subtyping of Adenovirus

NHRC has developed a multiplex-PCR assay to replace microneutralization as its current adenovirus typing method. This technique is currently undergoing validation testing. The benefits of PCR testing include cost-effectiveness and the elimination of dependence on type-specific antisera, which is not commercially available.

Retrospective Molecular Surveillance for Human Metapneumovirus and Rhinovirus

A new respiratory virus, human metapneumovirus (hMPV), was discovered in June of 2001. The clinical symptoms of hMPV are similar to those of human respiratory syncytial virus and include mild respiratory problems, cough, bronchiolitis, and pneumonia, often presented with high fever, myalgia, and vomiting. NHRC has found hMPV among FRI specimens that were negative for our standard respiratory panel. Intensive testing of banked specimens is underway, which will allow us to estimate the burden of hMPV in the recruit population. A manuscript is currently being developed.

Triangulation Identification for Genetic Evaluation of Risks

Ibis Therapeutics is developing a high-throughput, single-pass process for the simultaneous detection of a large number of infective threat agents, named Triangulation Identification for Genetic Evaluation of Risks (TIGER). The process uses PCR primers to amplify housekeeping genes that are conserved across species. The PCR product is run through a highly sensitive mass spectrometer, and each pathogen can be identified by a unique pattern. TIGER has recently been successful in identifying both adenovirus and *Streptococcus pyogenes* from specimens provided by NHRC. The TIGER technology was also successfully implemented on a selection of GAS isolates from the recent outbreak at the Marine Corps Recruit Depot in San Diego. A manuscript showing the strength of this technique as compared to classic molecular methods will be submitted to *Proceedings of the National Academy of Sciences* this month. Additional respiratory pathogens will be utilized to further develop this methodology.

Upcoming Protocols

Correlation between environmental sampling and adenovirus outbreaks among military recruits — slated to begin in late 2003 at 3 training centers, to be determined.

Surveillance for Community-Acquired Methicillin-resistant *Staphylococcus aureus* Isolates Among U.S. Military Personnel — human use protocols are currently under development, and this study is slated to begin in early 2004.

Presentations and Posters

Presentations & Posters at Recent Conferences

Annual DoD Global Influenza Surveillance Program Business Meeting, May 27-23, 2003, San Antonio, TX.

- ◆ NHRC staff gave presentations on FRI studies, PCR testing for influenza, and the ambient temperature specimen study.

Strengthening Influenza Pandemic Preparedness through Civil-Military Co-operation, May 9-11, 2003, St. Petersburg, Russia.

- ◆ Evaluation of PCR testing for influenza and adenovirus using ambient temperature specimens.
- ◆ Detection of influenza virus and adenoviruses by RT-PCR and melting curve analysis using LightCycler®
- ◆ Naval Health Research Center Respiratory Disease Laboratory.

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San Diego Epidemiology Research Exchange, April 25, 2003, San Diego, CA.

- ◆ Self-reported symptoms compared to laboratory diagnoses of pneumonia cases: data from a double-blind placebo-controlled vaccine trial in military trainees.
- ◆ Risk factors for community acquired methicillin-resistant *Staphylococcus aureus* (MRSA) in military trainees: review of an outbreak in San Diego, California, 2002.

International Symposium on Respiratory Viral Infections, December 5-8, 2002, Casa de Campo, La Romana, Dominican Republic.

- ♦ Ambient temperature specimen collection: evaluation of PCR tests for influenza and adenovirus.

Presentations & Posters at Upcoming Conferences

The Sixth Annual Force Health Protection Conference, August 11-17, 2003, Albuquerque, NM.

- ♦ An alternative method for classification of febrile respiratory illness rates at U.S. military basic training centers.
- ♦ Antibiotic resistance and emm-gene typing of clinical *Streptococcus pyogenes* isolates from U.S. military recruits.
- ♦ Pneumonia etiology compared to self-reported symptoms: data from a double-blind placebo-controlled pneumococcal vaccine trial in military trainees.
- ♦ Application of LightCycler® for the detection of influenza viruses and adenoviruses by fluorogenic RT PCR and PCR onboard a U.S. Naval aircraft carrier.

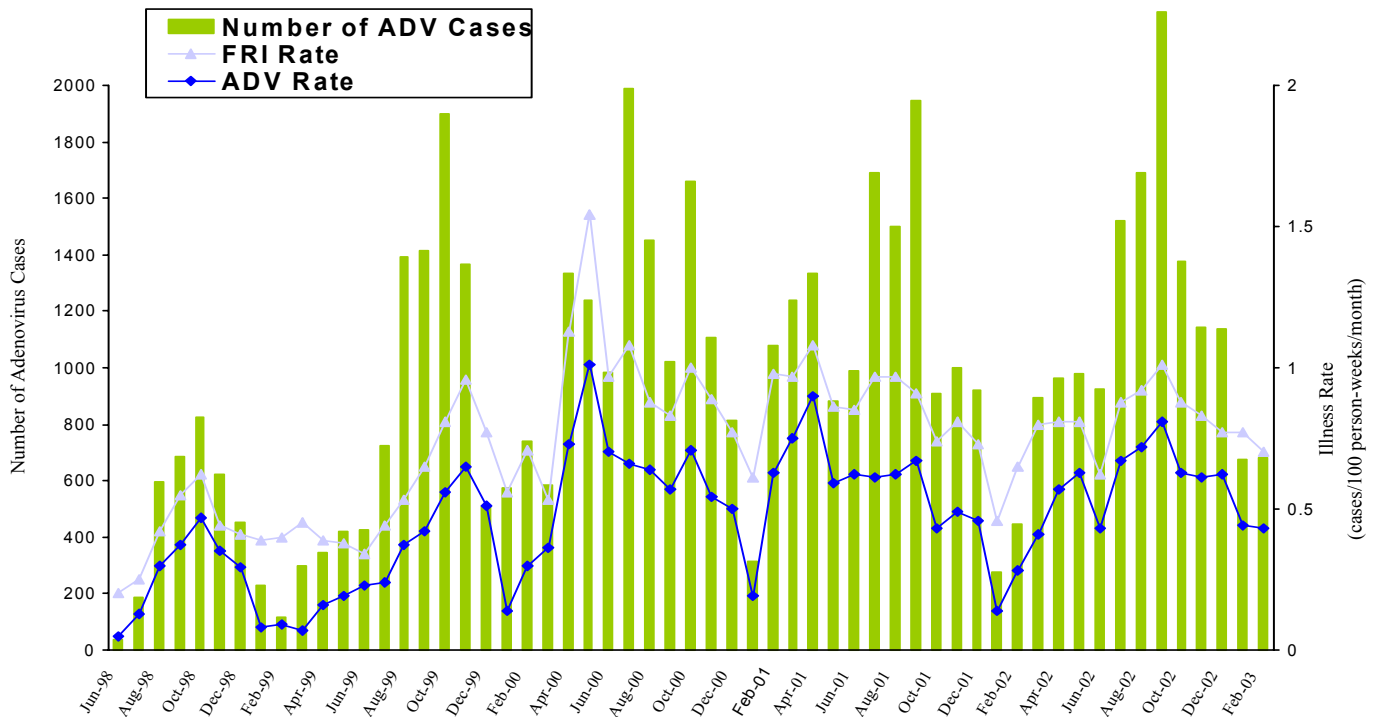
The Forty-First Annual Meeting of the Infectious Disease Society of America, October 9-12, 2003, San Diego, CA.

- ♦ Genetic analysis of adenovirus isolates from previously vaccinated military personnel.
- ♦ Descriptive epidemiology of febrile respiratory illness in a Mexican population.
- ♦ Multiplex polymerase chain reaction assay for detection of adenovirus in patient specimens.
- ♦ Molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates from community outbreaks in San Diego, California, 2002.

Please contact the newsletter staff with any questions, comments, or suggestions regarding the information in this issue.

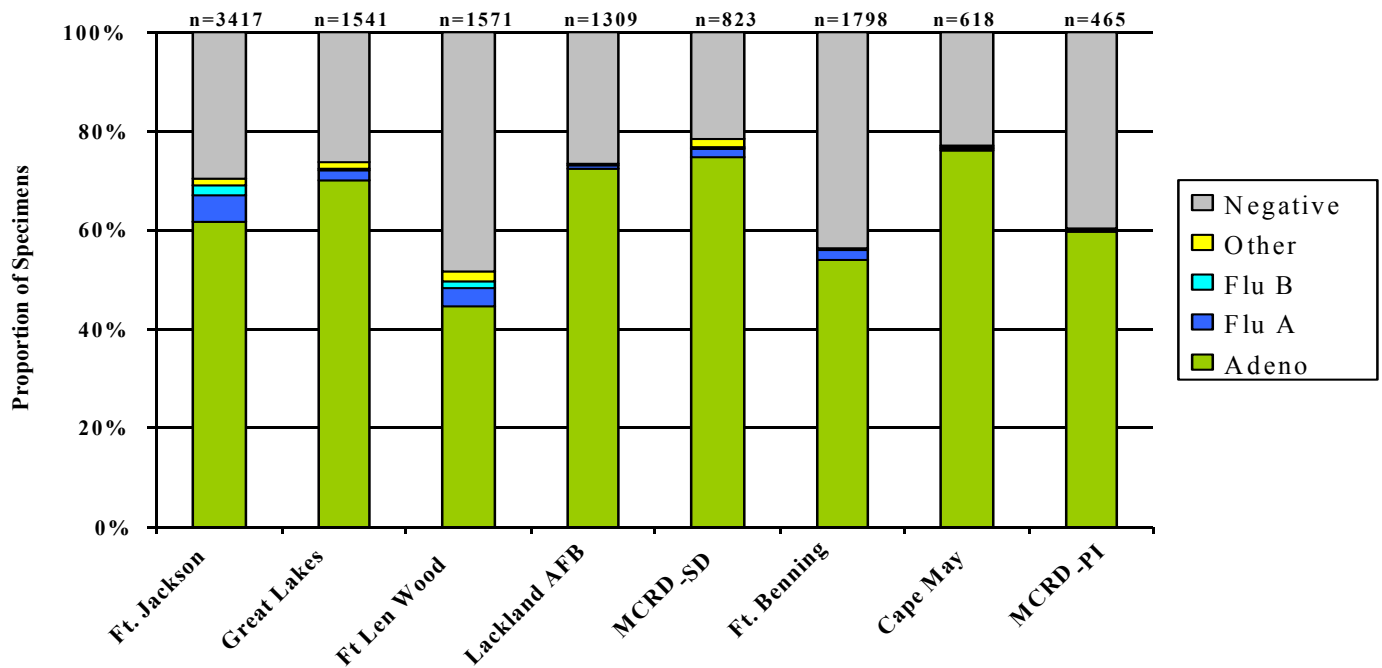
We would like to thank the personnel at our collaborating institutions for their hard work and dedication!

Combined Febrile Respiratory Illness (FRI) and Adenovirus (ADV) Morbidity Among Symptomatic Trainees at Eight Military Training Centers

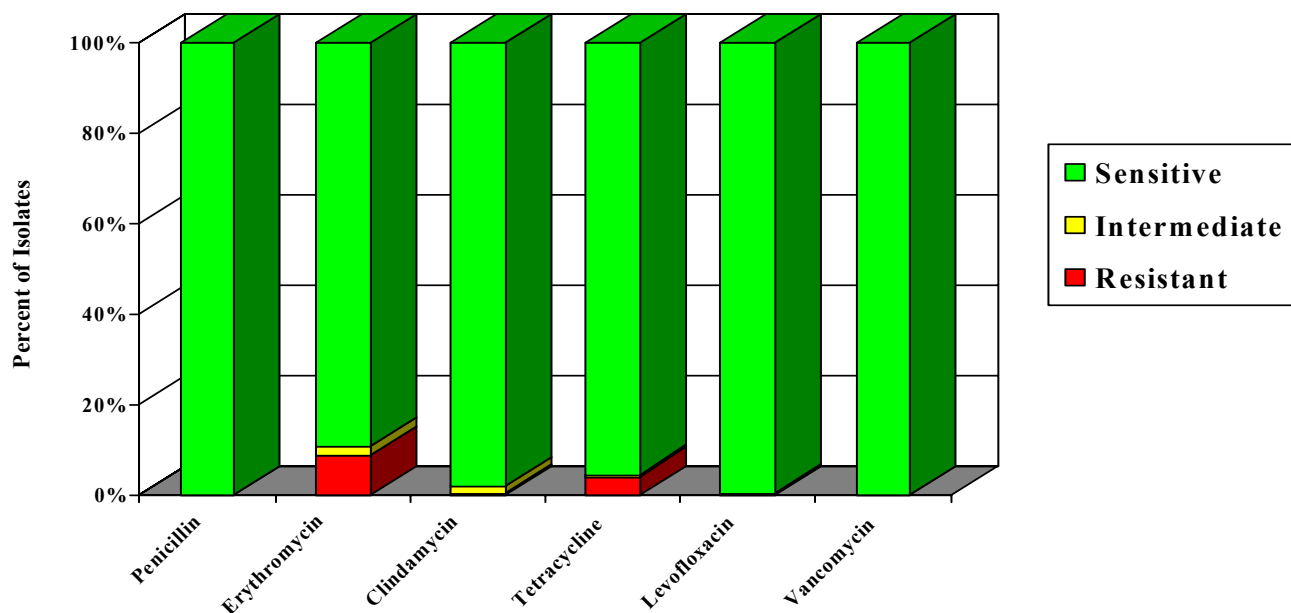


Distribution of Viral Test Results by Site

June 1998 - February 2003
n=11,542

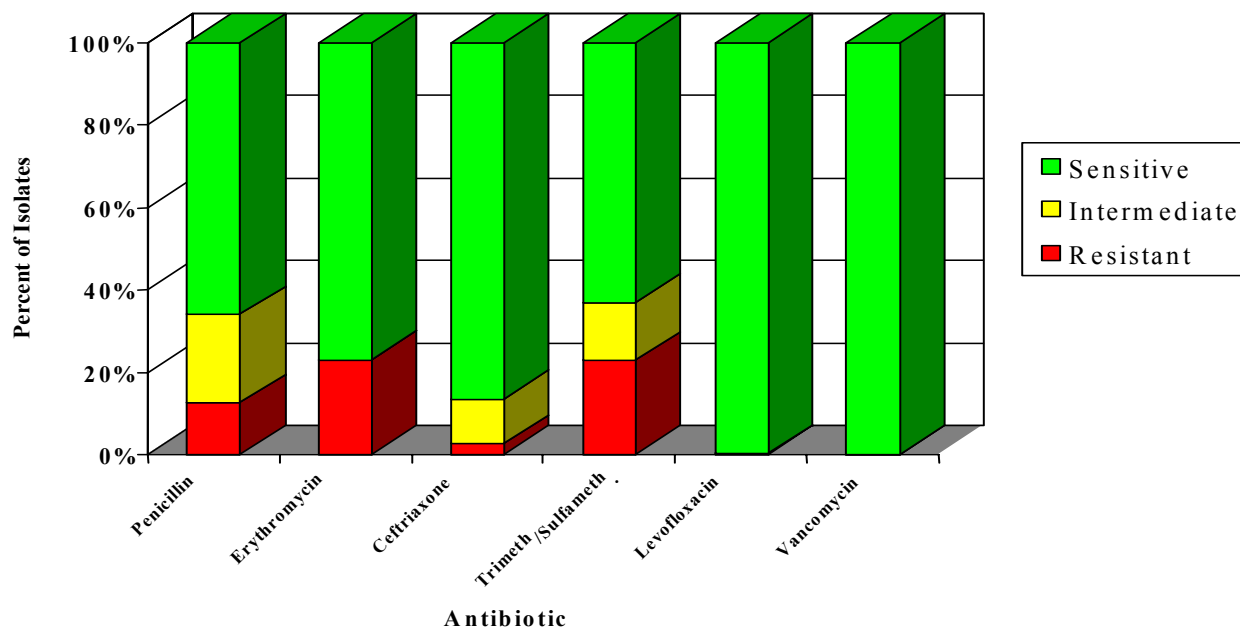


Antibiotic Resistance Patterns of Clinical *Streptococcus pyogenes* Isolates from Military Trainees



n=1297 isolates collected between 2/98 and 2/03

Antibiotic Resistance Patterns of Sterile Site *Streptococcus pneumoniae* Isolates From Military Medical Facilities



n=368 isolates collected between 8/97 and 12/02